Toxicology and Applied Pharmacology 316 (2017) 107–113

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/taap

Relationships among DNA hypomethylation, Cd, and Pb exposure and risk of cigarette smoking-related urothelial carcinoma

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ARTICLE INFO

Article history:
Received 21 September 2016
Revised 6 December 2016
Accepted 22 December 2016
Available online 24 December 2016

Keywords:
Cigarette smoking
Herbal medicine
Heavy metals
Global DNA methylation
Urothelial carcinoma

ABSTRACT

Cigarette smoking and environmental exposure to heavy metals are important global health issues, especially for urothelial carcinoma (UC). However, the effects of cadmium and lead exposure, as well as the levels of DNA hypomethylation, on UC risk are limited. We evaluated the possible exposure sources of Cd and Pb and the relationship among DNA hypomethylation, urinary Cd and Pb levels, and UC risk. We recruited 209 patients with UC and 417 control patients for a hospital-based case–control study between June 2011 and August 2014. We collected environmental exposure-related information with questionnaires. Blood and urine samples were analyzed to measure the Cd and Pb exposure and 5-methyl-2′-deoxycytidine levels as a proxy for DNA methylation. Multivariate logistic regression and 95% confidence intervals were applied to estimate the risk for UC. Study participants with high Cd and Pb exposure in blood or urine had significantly increased risk of UC, especially among the smokers. After adjusting for age and gender, the possible connections of individual cumulative cigarette smoking or herb medicine exposure with the increased levels of Cd and Pb were observed in the controls. Participants with 8.66%–12.39% of DNA hypomethylation had significantly increased risk of UC compared with those with ≥12.39% of DNA hypomethylation. Environmental factors including cigarette smoking and herb medicine may contribute to the internal dose of heavy metals levels. Repeat measurements of heavy metals with different study design, detailed dietary information, and types of herb medicine should be recommended for exploring UC carcinogenesis in future studies.

1. Introduction

The International Agency for Research on Cancer (IARC) recognized tobacco smoking as a class-1 carcinogen; sufficient evidence exists for it to be associated with urinary bladder cancer (Cogliano et al., 2011). According to the statistics of the Global Tobacco Surveillance System (GTSS) in 2015, the 879 million current tobacco users include 721 million males and 158 million females worldwide (Asma et al., 2015). Cigarette smoke is a complex chemical mixture containing more than 4000 identified chemical constituents, which include the environmental heavy metals cadmium (Cd) and lead (Pb). Cd and Pb might contaminate soil, accumulate in the tobacco plants, and become exposed to humans (Stellman and Djordjevic, 2009; Ceccaroli et al., 2015). Cigarette smoking and diet are the major sources of human exposure to Cd and Pb in the general population, except for the occupational exposure (Virani et al., 2016).

Cd, Cd-containing compounds and Pb have been classified as known human 1 and 2B carcinogens, respectively (IARC, 1987). Some epidemiological research showed that smokers had higher levels of Cd or Pb in the urine (Mohanty et al., 2015; Ruiz-Hernandez et al., 2015; Vilahur et al., 2015). Furthermore, the positive associations with chronic Cd exposure to the various cancers of the prostate, breast, lung, and bladder have been reported (Virani et al., 2016). Likewise, prostate and lung cancer have been associated with Pb (WHO, 1999; Tellez-Plaza et al., 2014). Previous studies about the acute effect of anemia, central nervous system, and joint and muscle pain for lead poisoning and pulmonary edema as well as respiratory tract irritation for Cd were available (Hernberg, 2000; Liu et al., 2009). The molecular mechanisms of the Cd- or Pb-induced carcinogenesis via reactive oxygen species (ROS) are well documented (Cogliano et al., 2011). However, studies on the

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http://dx.doi.org/10.1016/j.taap.2016.12.016
0041-008X/

effect of environmental Cd and Pb exposure in epigenetic-related carcinogenesis were presented. Global DNA methylation, which is one of the three epigenetic alterations, played an important role in carcinogenesis and is widely discussed. Recently, some indicators have been used to measure the levels of global DNA methylation, including measure the 5-methyl-2′-deoxycytidine (5-MedC) level on the whole genome and Alu or long interspersed nuclear elements-1 (LINE1) measurement (Chung et al., 2015). Jin et al. (2011) found a significant reduction of 5-hydroxymethylcytosine (5hmC) levels and the production of the oxidation product 5-MedC in the cancer patients as compared with the paired normal lung and brain samples (Jin et al., 2011). Significant DNA hypomethylation levels were also suggested in some cancer studies, including breast cancer, renal cell carcinoma, and bladder cancer (Moore et al., 2008; Kuchiba et al., 2014; Mendoza-Perez et al., 2016). However, other literature presented no associations between global 5-MedC and various cancer outcomes (Lee et al., 2008; Murata et al., 2013).

Previously, we found that the percentage of low 5-MeC levels was higher in invasive UC than in normal urothelia and non-invasive UC (Chung et al., 2015). However, the effect of DNA methylation on heavy metals-related UC carcinogenesis remains unclear. Therefore, we explored the interaction between the chronic, low-level, and non-occupational exposure to Cd and Pb via tobacco smoke or other potential factors and the global DNA methylation, which could increase the UC risk in our study.

2. Material and methods

2.1. Study participants

We conducted a hospital-based case-control study. The protocol of recruitment has been previously described in detail (Chung et al., 2014; Chang et al., 2016). Briefly, all the study participants, including 209 patients with UC and 417 control patients, were recruited from the China Medical University Hospital between June 2011 and August 2014. Patients with UC, aged 26–96 years, comprised the outpatients and inpatients of the Department of Urology, whose diagnoses were evaluated by a pathologist. In addition, we recruited the matched control participants with cases according to gender and age (±3 years) from among patients undergoing adult health examinations at the Department of Family Medicine. All the study participants signed the informed consent forms before receiving questionnaires, interviews, and urine specimen collection. The Research Ethics Committee of China Medical University Hospital in Taichung, Taiwan, approved the study (DMR100-IRB-080 and DMR100-IRB-262). Written informed consent was obtained from all study participants, and personal information was strictly protected.

2.2. Questionnaire and interview

Study participants were administered structural questionnaires and face-to-face interviews to acquire individual information on UC-related risk factors, including the demographics, socioeconomic characteristics, and lifestyle factors (e.g., cigarette smoking, alcohol drinking, herbal medicine use, and exposure to pesticides). For each subject, 10 mL of venous blood and 20 mL of spot urine were collected in Cd- and Pb-free containers, immediately transferred to a −20 °C freezer, and stored until further analysis. These specimens were individually used to measure the levels of global DNA methylation, Cd, and Pb in blood and urine.

2.3. Measurement of Cd and Pb in blood and urine

The concentration of heavy metals (Cd and Pb) in the blood and urine samples was measured in the Laboratory of Environmental Health and Occupational Medicine at the National Health Research Institutes by inductively coupled plasma-mass spectrometry (ICP-MS; Agilent 7700c,

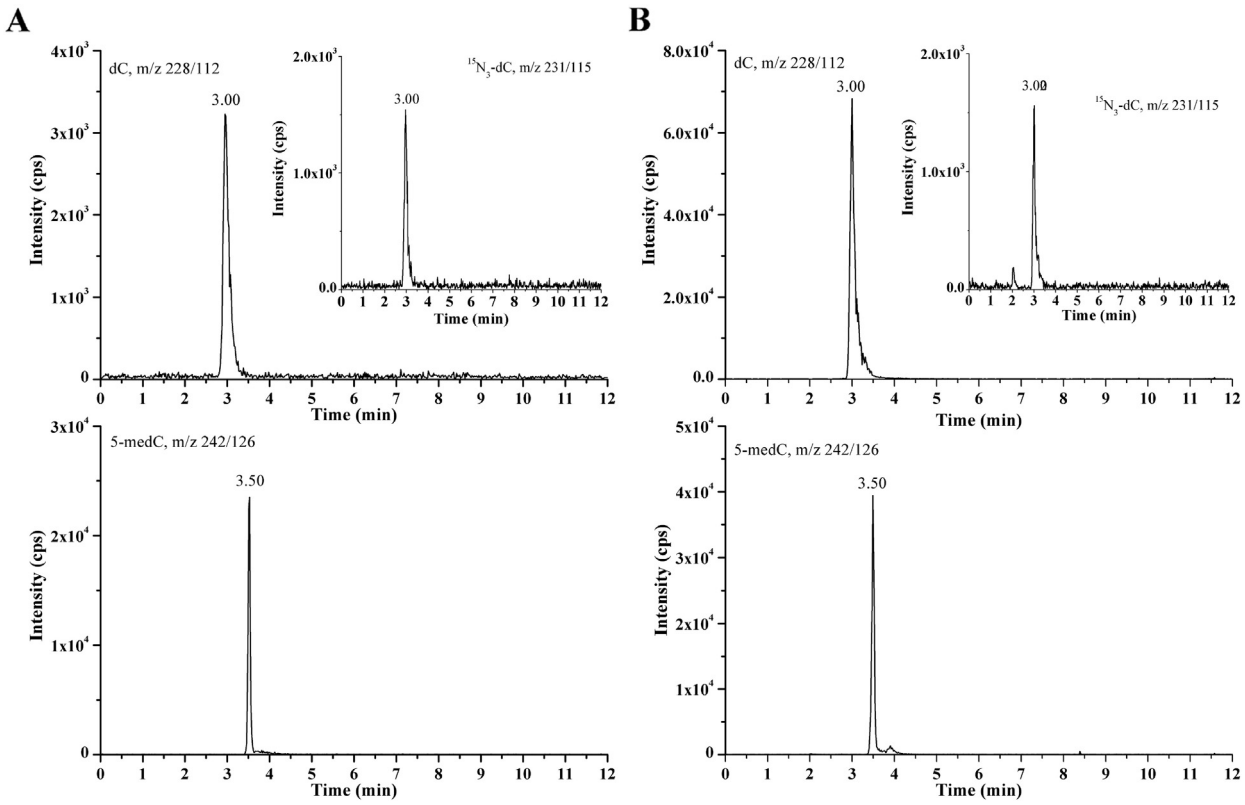

Fig. 1. LC–MS/MS chromatogram of reference compounds using MRM (multiple reaction monitor) mode. A: 10 ng/mL dC, 10 ng/mL 5-MedC, and 5 ng/mL $^{15}N_3$-dC; B: sample spike with 5 ng/mL $^{15}N_3$-dC. All compounds were dissolved in 5% MeOH + 0.1% FA.